The Pharma Innovation Journal 2023; 12(9): 770-775

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(9): 770-775

www.thepharmajournal.com
Received: 01-07-2023
Accepted: 05-08-2023

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Ensiling and rumen *in vitro* fermentation characteristics of whole-crop maize dough stage silage and maize stover silage harvested after grain maturity

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Abstract
Study was carried out in the Department of LPM, Veterinary College, Bangalore, with an objective to investigate the whether there is a possibility of converting maize stover into haylage or silage. The NAC 6004 maize variety grown in 0.1 ha was harvested manually, at 75 days (dough stage), at 100 days (grain harvest maturity). The fodder, thus harvested was chaffed and ensiled in three replicates in 10 liter polythelene boxes in a two (stage of harvest) x three (levels of treating) design, with three treatments *viz*., chaffed fodder as is (Control, C), C+ Jaggery at the rate of two per cent on as is weight (T1) and T1+Lactobacillus bacteria (LB) (Nutri-LAB ®, 600 Crores of spores per gram (Siddon Biotech Ltd, Banaswadi, Bengaluru) @ 100g per ton of feed) (T2). The results indicated that the quantity of fodder that could be packed in the plastic container ranged from 6.88 - 7.03 kg for maize dough stage silage (MdoS) and from 5.29 to 5.63 kg for maize stover silage (MstS). The weight of silages recovered the respective two silages ranged between 6.77 to 7.05 kg and 5.27 to 5.60 kg respectively. The mean recovery of biomass as silage for MdoS was 91.74 and for MstS was 96.58 %. The proximate compositions for whole plant maize fodder harvested at 75th day (MdoS) and for maize fodder after grain removal at 100th day (MstS) silages of different treatments were different only with respect to NDF and found same for rest of all the contents. The content of NDF was low in T1 when compared to other two treatments *viz*., C and T2. The relationship between both the control and treatment silages with additive was found to be significant (P =0.0108). The content of CP in MdoS was 8.31 and the same in MstS was 5.83 per cent. The difference was found to be non-significant. Lignin content was also found to be non-significant in MdoS (8.98) and in MstS (10.82). The gas production potential (*D* (ml/200 mg DM)) of C, T1, and T2 of MdoS and MstS were 99.66, 91.90, 85.10 and 66.28, 69.10, 68.54 respectively. The calculated ME (MJ/kg DM) content of MdoS (10.61) was found to be significant (P = 0.0220) when compared to MstS (8.13). The results of the current experiment indicates that similar to the green maize silage (MdoS), stay green maize stover may be used to make good quality silage with no addition of any additive. Whereas, addition of lactobacillus had an impact on the reduction in acceptability in animals.

Keywords: Maize, dough stage, stover, laboratory silage, chemical composition

Introduction
The residues obtained after crop harvest continue to remain as staple fodder for ruminant livestock in India. In spite of devoting considerable research and extension in improving crop residue utilization, significant amount of crop residues continue to remain poorly utilized resulting in wastage of as much as 60 to 70% depending on crop and feeding practices. For example, rice straw is burnt in rice cultivation regions to the extent of 80- 90%. Similarly maize straw (stover) in maize growing areas is burnt to the extent of 30-40%. The remainder although used as fodder, much of it is poorly utilized because of feeding in an un-chaffed form. It is also a practice to leave maize plants standing in the field after harvesting cobs, and allow to graze, wither or burn. In the process, a significant quantity of fodder is wasted by trampling, pest attack, leaching and decomposition as a consequence of unseasonal rain, or excessive dehydration due to heat and radiation (Ogunbosoye and Odedire, 2022) [12]. Through the process of conservation by ensiling fodder preserves nutritive components by decreasing pH by homo-fermentation of major soluble carbohydrates to lactate (Sun *et al*., 2009) [15] unlike in conventional drying under the sun that may lead to withering and loss of nutrients (Gouri *et al*., 2014) [6]. NRC (1988) [11] feed composition table reveals higher energy value in maize stalklage than in maize straw.

Ensiling green corn stover could significantly increase (p<0.01) the content of CP and Ash in DM of green corn stover. Furhter, ensiling corn stover increased (p<0.01) the DM, OM and NDF and also increased the disappearance rate of nutritive matter of green corn stover (Wang, 1999) [18]. The maize fodder variety has influence on nutritive value of silage was estimated for different silage. The corn stovers were harvested and conserved in the form of silage at the 104th day of growth. The chemical conents and nutritive value of silage was evaluated. Variety did not have a large effect on lactic acid and ADF content of silage with corn stover/plants. In this study the lactic acid concentrations and the digestibilities of organic matter *in vitro* of corn silage were higher than that of the silage of corn stalks. Results of trials indicate that the nutritive value of silage were significantly different with different variety (Mcdonald, P. (1981; Zhang, 2003; Yu Ruhua *et al*., 2007a) [9, 20, 19].

The nutritive value of maize (MS) silage and maize stover silage (MSS) was compared (Tang *et al*., 2008) [15]. The DM, OM, CP, NDF and ADF (%) for MS and MSS were 89.3 and 88.7; 93.3 and 91.3; 5.53 and 7.5; 76.6 and 67.1; and 48.9 and 41.4 respectively. Further the cumulative gas production at 48 h (ml) and potential gas (D) production for MS and MSS were 26.8 and 29.9 and 32.7 and 39.6 respectively. The results showed that the maize silage is more nutritive than the maize stover silage.

Therefore, it is hypothesized that maize stover conserved in wet form can have a better feeding value than the dried stover, especially for lactating cows in view of their demand for higher energy to meet the lactation needs. Therefore the current study was undertaken with the objective of converting maize stover fodder harvested soon after grain harvest into silage,

Materials and Methods

The experiment was done in the Department of LPM (Livestock Production and Management), Veterinary College, Bengaluru. Maize (NAC 6004) sown in 0.1 ha on 26-04-2010 by broadcasting and as per the agronomical recommendations given for the said variety for this study area. The area sown was made into sub-plots of 2 x 3 meter. The maize fodder grown under selected sub-plots was manually harvested, by leaving 5 cm from the ground level, at 75 days (dough stage) and at 100th days (after the grain formation for harvest). At 75th day, the maize grown maize fodder was harvested completely including cobs intact, whereas at 100th day, the maize fodder was harvested after grain or mature cobs removal. The entire harvested fodder was chaffed to a theoretical chop length of 1cm in a chop cutter machine (HIMCO-green fodder-6-8 tons/h and hay- 1 to 1.5 tons/h; C-5, Industrial Area, Clutter buckganj, Bareilly -243 301, UP, India). Chopped maize fodder was conserved in 3 replicas in 10 liter polypropylene boxes in a two (harvest stage) x three (level) factorial design. The stage of harvest were 75th and 100th day, and the treatment levels were chopped maize fodder as is basis (Control, C), chopped maize fodder added with 2 % of Jaggery as is basis (T1) and chopped maize fodder mixed with Jaggery @ 2% and LB (Nutri-LAB ®, spores with 6 billion numbers/g (Produred from Siddon ltd, Banaswdi, Bengaluru) at the rate of 100g per ton of feed) (T2).

The silage was prepared from a properly mixed fodder, by spreading 25 kg of chopped maize fodder on a PS (polythene sheet) to a thick layer of about 10 cm, with addition of 500 grams of jaggery and lactobacillus bacteria of 2.5grams was dissolved in 100 ml water and sprinkled all over on the fodder maize. Mixed or treated maize fodder was turned over several times to see that there was proper mixing of fodder with added additives. Jaggery and LB added or mixed maize fodder was then filled into polyethylene containers in 3 to 4 small portions through repeated compactions manually with hand and then by feet to eliminate air so as to create air free or no air remains in inside the containers or within the packed fodder. When filling of maize fodder was complete, the containers or boxes were placed with PS on over that a heavy stone (25 kg weight) was kept for 2 days to settle properly, under room temperature. To eliminate air getting trapped in the gap over the inside surface of cap was also filled with semisolid mud paste and finally the maize fodder was sealed with a PS and tightly compacted and closed with cap. Then the boxes or the containers were kept under normal environmental conditions (range 24 - 34 ^{o}C) for a period of eight weeks or 60 days.

The weight of empty container and the weight of container with fodder were recorded before closing the containers and after opening the containers. The samples of chaffed fodder and of silage were collected while filling the container and on opening the containers respectively for further analysis.

Sampling and palatability to animals: The containers were opened after 8 weeks of fermentation and representative samples taken from silages of the respective treatment groups in triplicates for laboratory or chemical analysis. The silages obtained from all boxes or containers were used for estimation of pH values and contents of dry matter and subjected to physical examination for physical properties. After dry matter estimation the representative specimens were preserved under normal environmental conditions for laboratory analysis and *in vitro* studies.

Remaining silage was fed randomly to two female cattle (under 1-2 year aged) kept on *ad libitum* green forage and with limited feeding of concentrate feed mixture (CFM), just to assess the acceptability of conserved maize fodder in the form silage, by physical noting of response towards the fed fodder.

Chemical analysis: The fodder specimens of green or as is basis fodder or MdoS and maize fodder after grain harvest or MstS were subjected for evaluation of dry matter contents (DM), crude protein (CP), ether extract (EE) and the TA (total ash) (AOAC, 1990) [2]. The Neutral Detergent Fiber (NDF), Acid Detergent Fiber and contents of lignin was estimated (Van Soest *et al*., 1991) [17].

Rumen *in vitro* gas production: Energy (ME) contents of MdoS & MstS was calculated through the *in vitro* studies (Menke and Steingass, 1988) [10]. The kinesis of production of gas was estimated (Krishnamoorthy *et al*., 1991) [8].

Rumen liquor procurement and maintenance of the cow donating it: Dairy cow with regular cyclical activity and under lactation yielding 12 kg milk/d, already fixed with a partially removable lid or rumen cannula of with bigger dia (Bar Diamond, Inc. USA), and this animal was given with a basal diet such as Maize stover silage and Concentrate feed mixture (CFM) (Maize-53, Wheat bran-41, Supplements of mineral-2, Salt-1, Urea-2 %) was utilized for rumen liquor collection (as the donor cow). Rumen fluid for *in vitro* studies was collected in the morning between 9.00 a.m. and 9.30 a.m.

Metabolisable energy (ME) determination: The ME in the rhodes hay, FMS and concentrate mixtures were determined by an *in vitro* gas production technique (IVGPT) (Menke and Steingass, 1988) [10] using the equations:

Concentrate supplements ME = 1.06 + 0.1570 GP + 0.0084 CP + 0.022 EE – 0.0081 TA

Roughages ME = 2.2 + 0.1357 GP + 0.0057 CP + 0.0002859 EE^2

Silage ME=13.99-0.0158ADF+0.0244EE

In this GP = gas production (milliliter/200 mgp on dry basis); CP=crude protein, EE= ether extract, TA=total ash (all in g/kg on dry basis).

Kinesis of production of gas: Air equilibrize maize fodder specimens (200 ± 10 mg) were loaded in measured glass syringe of capacity of 100 ml in three replicates according to Menke and Steingass, (1988) [10] each syringes were loaded with 30 ml of suspension of diluted fluid along rumen liquor, with 3 controls (blank incubations) and three graded (standard) syringes. The overall production of gas were taken at two, four, six, eight, ten, 12, 18, 24, 36, 48, 72, and 96hrs of incubation. The rate of gas production and extent of gas production was estimated by non-linear regression by using the model $Y = D (1 – e^{-k*t})$ where, Y is gas volume (ml) at time t, D is nothing but potential gas production (ml) and k is the rate (per hour) at which gas being produced (Krishnamoorthy *et al*., 1991) [8]. The time at half asymptotic gas production ($t_{1/2}$) was determined as ln2/k. Gas production at twenty four hour, corrected for the blank and standards and was used for estimation of Metabolisable energy (ME) as mentioned above.

Statistical analysis: The experimental data were analyzed using analysis of variance (ANOVA) technique (Snedecor and Cochran, 1968) [13].
Graphpad prism 4 for (2019) software for *in vitro* studies (RIVGP) gas kinesis, windows SPSS for (2023) Statistics 17.0. Inc., software was used to analyze for all statistical analyses.

Results and Discussion

Physical Appearances or characteristics: Colour, odour, appearance of mold formation, and weight in kilograms for the packed maize fodder quantity in the boxes, once they were sealed for fermentation and opened after fermentation (8 weeks) for both the silages which were then added with additives represented in Table 1. The fodder conserved in the form of silages on as is basis or dough-stage and after grain harvest or maize stover were having green- yellow and yellowish-green respectively. The silage was having a sweet smell and the similar result was found in all the treatments for both silages and haylages. In both the silages no visible fungal growth was seen. The quantity of fodder that could be put in the boxes or container ranged from 6.88 to 7.03 kg for whole maize as is basis or MdoS and from 5.29 to 5.63 kg for MstS. The quantity of silage obtained for the consonant, either the silage or haylage varied from 6.77 to 7.05 kg and 5.27 to 5.60 kg respectively. The mean recovery of biomass as silage for the two types of fodder were 91.74 and 96.58 per cent respectively.

Chemical composition and rumen fermentation characteristics: The pH, dry matter content, chemical or proximate composition and rumen RIVGP kinetics of both the silages with or without addition of additive treatments are represented in Table 2. Irrespective of types of silages and also addition of additives, the pH ranged between 3.60 - 3.96 and the variation in the pH for silages (P = 0.1835) and between the additives silage (P= 0.1352) was found to be non-significant. The average dry matter (DM) contents of maize fodder used for MdoS was 26.0 per cent. On the other hand the maize fodder used for MstS was forty one per cent. The dry matter contents of both the silages were 24.04 and 39.91 per cent. The variations in contents of dry matter in fodder used for silage were found to be statistically significant (P =0.0029). But the additive treatment silages had non-significant effect on DM content of silage.
The chemical or proximate contents of compositions of both the of silages, which was added with different additives was found to be same for all the values but was varied with respect to NDF. The average values of NDF for Mdos was 61.64 and for MstS was 64.82% (P =0.014). Lowest Neutral detergent fibre was seen in T1 compared to control and treatment 2 silage. The average crude protein (CP) contents of MdoS was and MstS were 8.31 and 5.83 per cent respectively, but the variation was non-significant. Even lignin contents in MdoS was 8.98 and in MstS 10.82 per cent was also found to be non-significant.

Rumen *in vitro* gas production kinesis: Rumen fermentation production of gas pattern for the two types of silages added with additives to are shown in Figure 1. Production potential gas (*D* (milliliter/200 mg on dry basis) of T1, T2 and T3 from MdoS and MstS were 99.66, 91.90, 85.10 and 66.28, 69.10, 68.54 respectively. Similarly the k for the corresponding silage were 0.03737, 0.04869, 0.04182 and 0.04182, 0.40155, 0.0409 respectively and the difference among the silage was not significant. The estimated ME (MJ/kg on dry matter basis) contents of MdoS (10.61) was found to be significantly (P = 0.0220) higher when compared to MstS (8.13).
The silage prepared from whole maize fodder harvested at early or dough stage and after grain harvest or stover physically appeared similar without any mold growth. There was a similarity in both colour and smell of both the silages of MdoS and MstS. It was further substantiated by the normal pH values. The pH values of silages irrespective to their stage of harvest and additive treatment were within a normal range from 3.6 to 3.96. Seepage or loss due to seepage was not observed in any of the boxes or containers and the dry matter recovery was 92.0 % with MdoS and 96.0 % in MstS respectively. The indication of no seepage and normal recovery of dry matter are the important parameters which indicates that there was satisfactory anaerobiosis and fermentation took place in the boxes. If the packing of fodder was not proper may lead loss of dry matter due to oxidation through presence of air leading heat formation. The solidity (kilograms per cubic feet) of maize fodder kept in boxes was 15.0 for MdoS and 12.0 for MstS. This is in accordance with

recommendations (John Moran, 2005 and Arieli *et al*., 1998) [7, 4]. The lower dry matter recovery in MdoS could be indicative of higher volatile substances in MdoS. Although total volatile substances was not determined in this study, since maize fodder at dough stage is expected to contain more fermentable carbohydrates than the stover, higher amount of volatile fermentation end products can be expected. Higher level of neutral detergent soluble discounted for ash, protein and ether extract and higher D in MdoS (92.22) compared to MstS (97.99) lends support to this assumption. The report of (Arias *et al*., 2003: Arieli *et al*., 1998) [3, 7] revealed that from the nutritional point of view, the degradable substrate provided by the stover will depend on the contribution of two different pools. One is soluble fraction content that is soluble carbohydrates presence, which depends on the amount of soluble carbohydrates stored in stover less the fraction lost by respiration and drip off during conservation or ensiling and subsequent storage. The other that is the second pool which is the insoluble but degradable fraction can be degraded by ruminal micro-organisms during the time (stover degradation by 24 h) that silage is retained in rumen. In general, the lower level of fermentable carbohydrate content in maize fodder used for silage after grain removal and to prevent this problem, addition of fermentable sugars *viz*., molasses or floor of non-legumes or millet grains or maize grains floor and culture of microbes and supplementation of yeast cultures may be used hasen the fermentation process. However, the pH of MstS being similar to that of MdoS in control suggests that the lower carbohydrate which are fermentable that are usually found in maize stover has not limited the fermentation process in this study. This is further evident from a lack of difference among additive treatments for both MdoS and MstS. Palatability observation also proved that there was readily acceptance of control and T1 of both the silages. But there was disinterest shown for silages for treatment T2.

Table 1: Physical properties, weight changes, dry matter content and pH in both silages with addition of supplements

Parameter	**Types of silages**						**P - Value**		
	MdoS			**MstS**					
	C	**T1**	**T2**	**C**	**T1**	**T2**	**Silages**	**Additive**	**Inter action**
Physical properties									
Colour	Greenish yellow	Greenish yellow	Greenish yellow	Yellowish green	Yellowish green	Yellowish green	-		
Smell	Sweet	Sweet	Sweet	Sweet	Sweet	Sweet	-		
Mold Formation visibility	-	-	-	-	-	-	-		
Weight (kg) of fresh matter							-		
Fodder	7.03±0.15	7.03±0.08	6.88±0.19	5.29±0.03	5.46±0.04	5.63±0.03	NS	NS	0.047
Silage	6.99±0.13	7.05±0.26	6.77±0.17	5.27±0.02	5.42±0.03	5.6±0.03	NS		
Weight (kg) of dry matter									
Fodder	1.83	1.83	1.79	2.17	2.24	2.31	0.049	NS	NS
Silages	1.67	1.69	1.64	2.10	2.16	2.23	0.001	NS	NS
Dry matter %									
Fodders	26	26	26	41	41	41	0.0001	NS	NS
Silage	23.71	23.57	24.83	39.73	39.67	40.33	NS	NS	0.008
pH	3.96±0.05	3.83±0.04	3.80±0.01	3.86±0.04	3.7±0.03	3.6±0.03	NS	0.009	0.047

MdoS -Maize fodder dough stage silage, MstS- Maize stover silage C- Control, T1 - Jaggery20 g/kg of fodder, T3 - Jaggery20 g/kg of fodder + Lactobacillus culture @ 100mg/kg of fodder, NS- Non-significant

Table 2: Chemical composition, rumen *in vitro* gas production, kinetics (potential gas production (D, ml/200mg DM), rate of gas production (k h^{-1}) in both silages with addition of supplements

Parameter	**Types of silages**						**P- Value**		
	MdoS			**MstS**					
	C	**T1**	**T2**	**C**	**T1**	**T2**	**Silages**	**Additive**	**Interaction**
DM	92.66 ±0.19	92.44 ±0.35	93.04±0.09	93.35±0.30	92.82±0.29	92.87±0.29	0.001	0.052	0.017
OM	93.18±0.17	92.97±0.44	93.55±0.19	93.46±0.34	94.09±0.38	93.33±0.38	NS		
CP	8.43 ±0.26	8.31±0.14	8.20±0.15	6.07±0.14	5.71±0.18	5.72±0.18	NS		
NDF	61.54 ±1.66	60.18±2.23	63.19±3.96	66.96±2.71	61.50±0.82	66.01±0.82	0.014	0.011	0.0108
ADF	41.29 ±0.96	38.03±1.28	40.74±0.96	49.57±2.12	45.03±2.04	53.19±2.04	NS	NS	0.001
EE	1.85 ±0.06	2.10±0.09	1.96±0.03	1.63±0.03	1.87±0.03	1.33±0.03	NS		
TA	6.82 ±0.05	7.03 ±0.14	6.45±0.14	6.54±0.28	5.91±0.13	6.67±0.13	NS		
Lignin	8.48 ±0.53	8.06±.39	10.41±0.26	10.86±0.41	9.94±0.34	11.67±0.34	NS		
RIVGP-24h (ml/200mg)	58.56	60.94	53.44	38.83	42.06	41.22	0.0191		
ME (MJ/kg DM)	10.72	11.07	10.03	7.89	8.33	8.17	0.0220		
D (ml)	99.66	91.90	85.10	66.28	69.10	68.54			
k (h^{-1})	0.03737 (0.9462)	0.04869 (0.9773)	0.04182 (0.9945)	0.04182 (0.9726)	0.40155 (0.9598)	0.04090 (0.9697)			

MdoS -Maize fodder dough stage silage, MstS- Maize stover silage C- Control, T1 - Jaggery20 g/kg of fodder, T3 - Jaggery20 g/kg of fodder + Lactobacillus culture @ 100mg/kg of fodder, NS- Non-significant

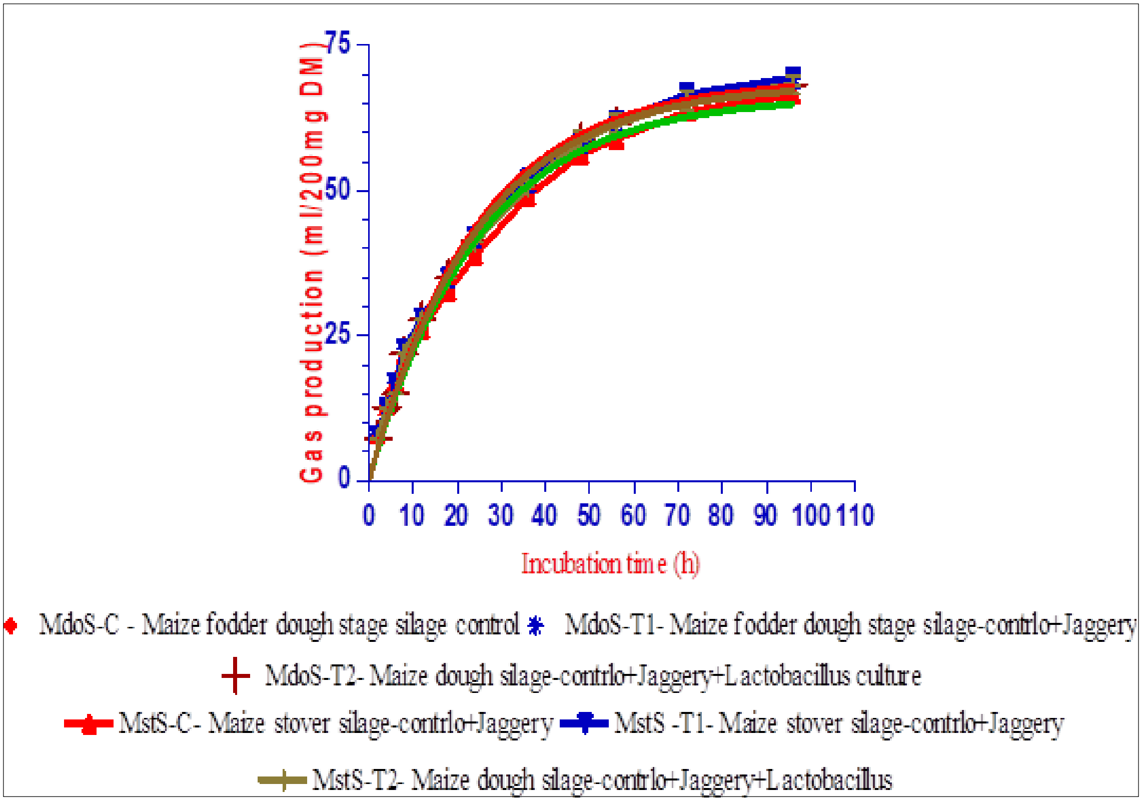

Fig 1: Rumen *in vitro* gas production from two type of silage subjected to addictive treatments

Conclusion

From the above findings it was concluded that both green or MdoS and wet maize stover harvested soon after grain removal or MstS can be easily stored in the form of good quality of acceptable form of silage without any additives and addition of lactobacillus.

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